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Semi-automated detection of fractional shortening in zebrafish embryo heart videos

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Abstract: Quantifying cardiac functions in model organisms like embryonic zebrafish is of high importance in small molecule screens for new therapeutic compounds. One relevant cardiac parameter is the fractional shortening (FS). A method for semi-automatic quantification of FS in video recordings of zebrafish embryo hearts is presented. The software provides automated visual information about the end-systolic and end-diastolic stages of the heart by displaying corresponding colored lines into a Motion-mode display. After manually marking the ventricle diameters in frames of end-systolic and end-diastolic stages, the FS is calculated. The software was evaluated by comparing the results of the determination of FS with results obtained from another established method. Correlations of $0.96 < r < 0.99$ between the two methods were found indicating that the new software provides comparable results for the determination of the FS.

Keywords: fractional shortening; heart; semi-automatic quantification; zebrafish.

1 Introduction

Over the past 25 years, the zebrafish has become a model organism to study cardiogenesis and heart diseases, for fundamental research as well as other biomedical applications [1–4]. Due to the transparency of zebrafish embryos throughout embryogenesis, a real time *in-vivo* observation of physiological or pathological events occurring during organ development is possible [4, 5, 6]. Determination of cardiac contractility is useful to elucidate cardiac and circulation defects in zebrafish embryos. Fractional shortening (FS) measurement has been shown to be a simple and fast method to determine the contractile force of embryonic zebrafish hearts. The transparency of the embryos allows to easily capture videos of the beating heart [6], which then enables the measurement of the systolic and diastolic diameters. After the determination of these diameters, *FS* is calculated by the following formula:

$$FS = \frac{\emptyset diastole - \emptyset systole}{\emptyset diastole} \quad (1)$$

Image processing has increasingly become a powerful approach leading to the continuous development of automated methods that help to quantify and analyse many cardiac parameters of interest [4–9]. In fact, automated analysis methods are of great value as they can drastically accelerate the evaluation of heartbeat parameters such as frequency, beat-to-beat intervals, and arrythmicity [6]. Unsurprisingly, many image- and video-based studies of zebrafish require the build-up of custom video analysis software for various domains of research and experiments, allowing flexible and quick handling and processing of input and output data [6, 9]. For instance, high throughput screenings of zebrafish as needed in small molecule evaluations, toxicological experiments and behavioural analysis is only made possible with custom video analysis software [9]. Using this factual background about the importance and advantages of customizing software applications for image analysis [6, 9], the development of a reliable, semi-automated detection method of FS was made possible.

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2 Methods

2.1 Procedure and materials

The algorithms used to semi-automatically determine the FS are implemented in Microsoft Visual Studio Community 2015 with C# using the .NET Framework 4.5.2. Additionally the image processing-related classes and functions were composed using the AForge.NET framework. This approach allowed us to develop a stand-alone application capable of being easily deployed to a large number of computer systems as well as delivering sufficient performance even on standard office computer hardware.

Videos of embryonic wild type zebrafish hearts with various resolutions (from 288 × 256 up to 1392 × 1056 pixels) and frame rates (between 7 and 100 frames per second) as well as different video codecs (Motion JPEG, MPEG-4 (DX50) or H264/MPEG-4 AVC) were used to test the software.

2.1.1 Determination of the end-diastolic phase

The heart repetitively changes its shape during systole and diastole with a short end-diastolic rest. This rhythmically altering of shape can be distinguished automatically by means of image analysis, if the frame rate of a video of a beating heart is several times the heartbeat frequency (e.g. >30 frames per second for zebrafish embryos). The similarity coefficient between two consecutive images can be calculated by first summing up the absolute pixel value differences between two consecutive images in both h and w direction and then dividing them by the maximum pixel byte value, 255 multiplied with the total pixel count. This process is referred to as a normalized sum of absolute differences (SAD). For 8 bit grayscale images the similarity coefficient can be described by equation 2, while for RGB images every colour channel needs to be taken into account.

$$c_s = 1 - \frac{\sum_{i=0}^{h} \sum_{j=0}^{w} |b_{s(i,j)} - b_{t(i,j)}|}{255 \times h \times w} \qquad (2)$$

where h is the image's height, w is the image's width in pixels, b_s is an image, b_t is the consecutive or template image and 255 is the standard maximum byte value.

The similarity coefficient between two consecutive end-diastolic images will be higher (closer to 1) than between two subsequent images where the heart shape changes quickly and the ratio for the similarity coefficient is closer to 0. By choosing consecutive pairs of images with the highest similarity coefficients the end-diastolic phase can be determined automatically. When comparing every individual frame with the subsequent one in the video frames series, a uniform pattern of oscillation occurs, as depicted in Figure 1.

If the frame rate of the video is too low (e.g. <20 frames per second for zebrafish embryos) the automatic determination of the end-systolic and end-diastolic phase by calculation of the similarity coefficient does not work reliably. In this case the number of frames between every transitional instance of the heart beat cycle is too small to locate the end-systolic and diastolic stages using this kind of minimum values. In order to refine the large set of video frames to only those showing the end-systolic and the end-diastolic stages of the heart beat cycle a single template frame has to be selected manually. This is supported by a graphical user interface where a selected video recording of the heart is accessed by the DirectShow interface. All video frames are temporarily stored in memory for further processing and displayed to the user. The first most likely frame pertaining to the end-diastolic stage of the heart beat cycle has to be chosen by the user. This template image is then used for a template matching algorithm, where the selected template image is compared to all other frames of the video for its similarity coefficient, as given in equation 2. The maxima values represent the frames being most similar to the selected template frame and correspond to frames of the same end-diastolic phase of heartbeat. Furthermore, the frames least similar to the template frame deliver minima values for the similarity coefficient and denote the end-systolic phase.

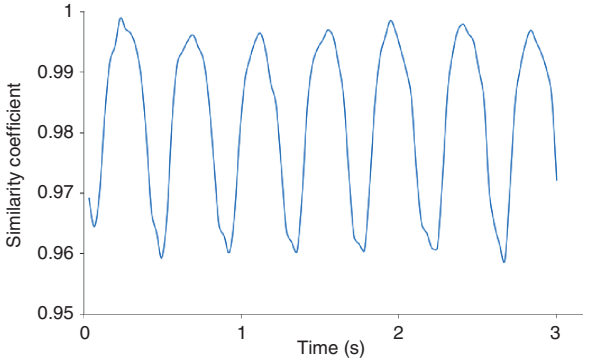

Figure 1: Plot of the similarity coefficients for a heart video. Lower similarity is found during rapid changes of the heart shape and high similarity during end-diastole.

2.1.2 M-mode Plot

Subsequently, the patterned change in the shape of the heart throughout the cycle is displayed in an M-Mode, as given in Figure 2.

The M-Mode is generated by the excision and alignment of 1-pixel-wide, vertical strips from successive video frames. Maximum contraction (end-systole) and relaxation (end-diastole) of the heart ventricle can be more readily viewed at once. Results of the automatically determined end-systolic and end-diastolic frames are also plotted as vertical lines in red (end-diastolic) and blue (end-systolic) and green for the current frame that is displayed for marker setting. Each strip lies at the middle of its corresponding frame passing through the centre of the heart [7, 10]. Hereafter, only the end-systolic and end-diastolic frames are displayed to the user to navigate through, excluding the other frames, because they are of no obvious significance for the user anymore.

2.1.3 Calculation of the fractional shortening

To calculate the FS percentage, it is necessary to have readings of the end-systolic and end-diastolic heart diameters. This is obtained by manually marking the heart edges during end-diastole or end-systole. Two pairs of marking points identifying the diastolic heart edges and two pairs identifying the systolic heart edges have to be set. In each marked frame, the two pairs of markings give length readings for two lines. One line is drawn across the longest segment of the heart, and the other is much shorter, almost perpendicularly drawn (Figure 3). By marking the heart edges of one pair or several equal numbers of end-systolic frames and end-diastolic frames, an average value of the FS for the large diameters and another average value for the small diameters are obtained. This way the interface outputs two average values for the FS percentage. In addition, the standard deviation of the calculated FS averages is displayed.

3 Results

The software saves the output in an Excel® file, along with information about the x and y coordinates of the marked points, diameter readings, frame numbers, etc. This way, the data for each video can be easily accessed at any time. The software was evaluated by comparing the results provided from manual analysis by an experienced biologist with those obtained with the software. First the FS of 15 embryonic zebrafish hearts were determined by one author by manually placing markers onto maximum ventricular systole and ventricular diastole frames, comparable to the method in [2]. The FS of the 15 zebrafish hearts were in the range of 32 and 70% (see Figure 4).

Afterwards the new software was used by two of the other authors independently from each other to determine the FS of the same zebrafish videos. The FS determined by the second and third examiner were in the range of 30–72% and 37–69%, respectively. Pearson's linear correlation coefficients between the results of each of the examiners were calculated. Correlations of $0.96 < r \quad 0.99$ for all cases indicate that the new software provides comparable results for the determination of the FS with the benefit of increased speed and ease of use.

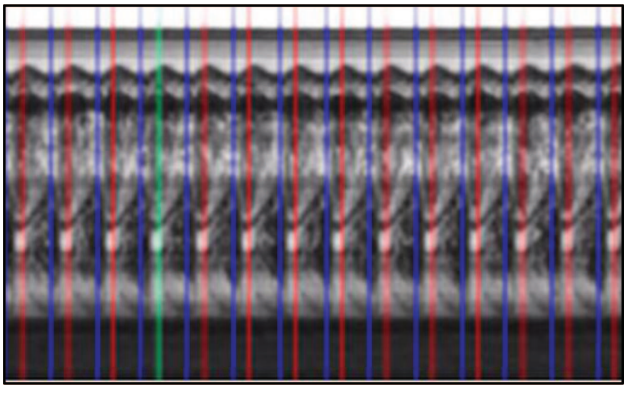

Figure 2: M-Mode, displaying changes in movement of the heart edges (pixel number in y-axis) over time (frame number in x-axis). Automatically computed end-systolic and diastolic frames are also plotted as vertical lines in red (end-diastolic) and blue (end-systolic).

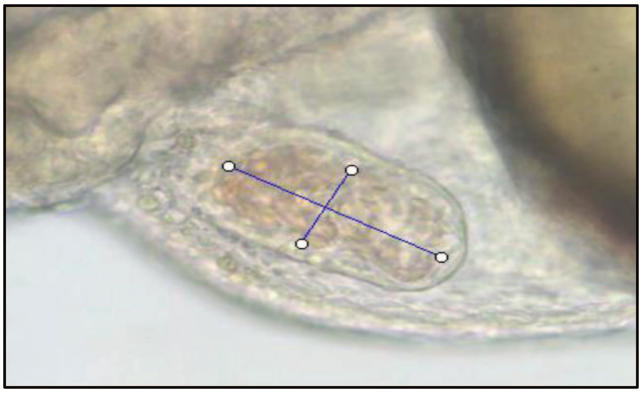

Figure 3: A pair of lines marking the ventricle's small and large diameters.

4 Conclusion

Evaluation of FS is important to determine heart ventricle and atrium function in order to calculate the ejection fraction. Zebrafish can be used as model organism to investigate small molecules for their suitability as potential cardiac drugs. A new software was presented that can accelerate the determination of the FS in zebrafish heart videos by identifying and presenting only those frames, which have been identified as corresponding with the end-systolic and end-diastolic phase. The new method has three steps: (i) Automatic or manual selection of a template frame (ii) Marking of the heart edges to identify end-systolic and diastolic heart diameters for several frames; (iii) Calculation of the average FS and the standard deviation of the set of readings. Videos can be processed independent of rhythmicity. For instance, cardiac arrhythmia should not affect the working of the algorithm as it is independent on the number of frames between each status frame. Similarly, heart conditions which involve absence of blood are not a problem in detection as it is not based on the colours in the frame, but rather the heart shape.

The software has proved to deliver results for the FS of videos from zebrafish embryos that correlated with the results obtained by a standard manual method.

Additionally, there are indications that the new software can also been used to evaluate human cardiac ultrasound videos. The software has been tested with such videos that were provided at a video-sharing website [11–13]. Again, the results for the FS were in the same range as given in the videos.

In addition, having the ability to input and process many readings from which the average FS percentage and its standard deviation are calculated gives the results a statistically powerful property, which improves robustness and reproducibility of the detection. This helps in future trials to process larger sets of data reliably, as well as in developing a fully automated system.

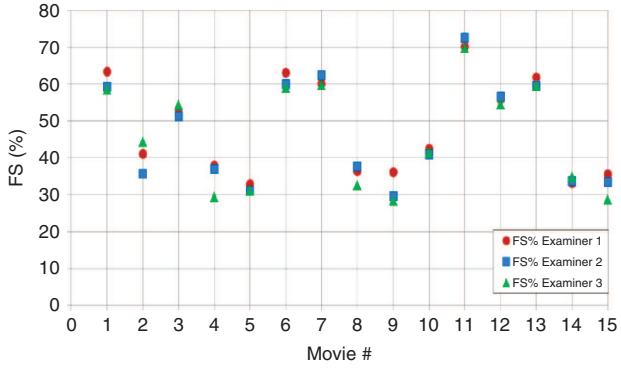

Figure 4: Results of the determined fractional shortenings (FS) from three examiners. The first examiner used a conventional method as a reference as described in [2].

The new software is available on request by the corresponding author.

Author's Statement

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References

[1] Yang L, Ho NY, Alshut R, Legradi J, Weiss C, Reischl M, et al. Zebrafish embryos as models for embryotoxic and teratological effects of chemicals. Reprod Toxicol. 2009;28:245–53.
[2] Hoage T, Ding Y, Xu X. Quantifying cardiac functions in embryonic and adult zebrafish. cardiovascular development. Humana Press; 2012;843. p. 11–20.
[3] Bakkers J. Zebrafish as a model to study cardiac development and human cardiac disease. Cardiovasc Res. 2011;91:279–88.
[4] Bührdel JB, Hirth S, Keßler M, Westphal S, Forster M, Manta L, et al. In vivo characterization of human myofibrillar myopathy genes in zebrafish. Biochem Biophys Res Commun. 2015;461:217–23.
[5] De Luca E, Zaccaria GM, Hadhoud M, Rizzo G, Ponzini R, Morbiducci U, et al. ZebraBeat: a flexible platform for the analysis of the cardiac rate in zebrafish embryos. Scient Reports. 2014;4898:1–13.
[6] Mikut R, Dickmeis T, Driever W, Geurts P, Hamprecht FA, Kausler BX, et al. Automated processing of zebrafish imaging data: a survey. Zebrafish. 2013;10:401–21.
[7] Fink M, Callol-Massot C, Chu A, Ruiz-Lozano P, Izpisua Belmonte JC, Giles W, et al. A new method for detection and quantification of heartbeat parameters in drosophila, zebrafish, and embryonic mouse hearts. Biotechniques. 2009;46:101–13.
[8] Pylatiuk C, Sanchez D, Mikut R, Alshut R, Reischl M, Hirth S, et al. Automatic zebrafish heartbeat detection and analysis for zebrafish embryos. Zebrafish. 2014;11:379–83.
[9] Marcato D, Alshut R, Breitwieser H, Mikut R, Strahle U, Pylatiuk C, et al. An automated and high-throughput Photomotor Response platform for chemical screens. Conf Proc IEEE Eng Med Biol Soc. 2015;7728–31.
[10] Ocorr K, Vogler G and Bodmer R. Methods to assess Drosophila heart development, function and aging. Methods. 2014;68:265–72.
[11] http://youtu.be/izdhV1Owfrk.
[12] http://youtu.be/K8EUvn1NSY8.
[13] http://youtu.be/ZuKFy3Z6.